Energetiq Soft X-Ray Light Source

EUV Litho 2011

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Outline

- History and background
  - Motivation
  - Recent published results
- Source development
- Demonstration microscope
- Integration with commercial microscope; imaging results
- Conclusions

Soft X-Ray Biological Imaging

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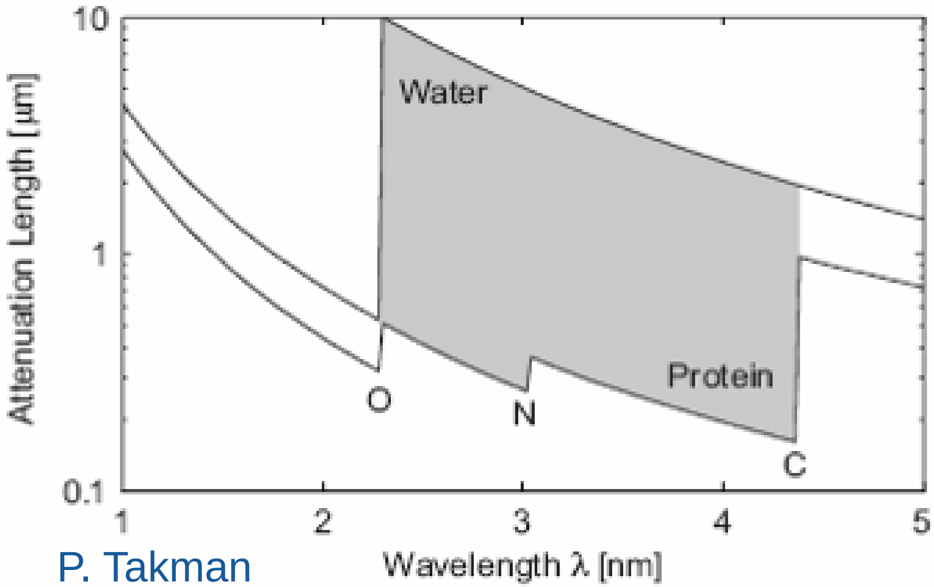
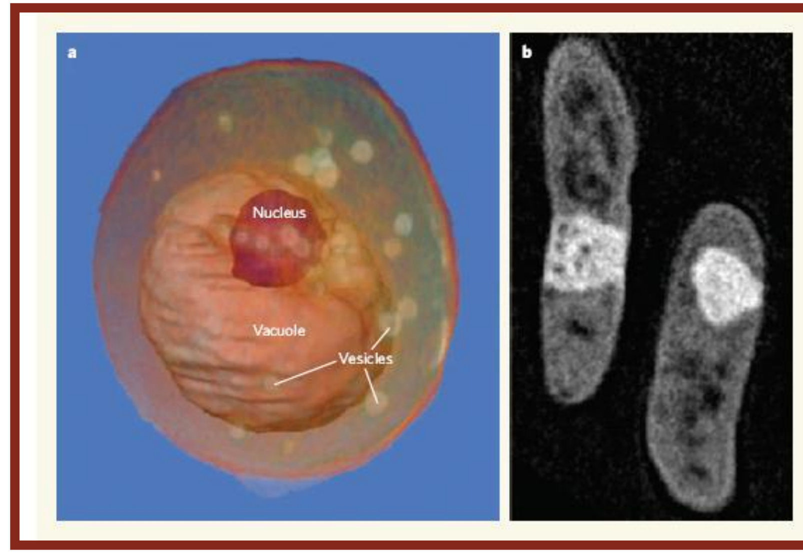


P. Takman

- High resolution x-ray images of the inner structures of biological cells w/o staining
- Tomography gives quantitative results
  - 3-d density map
  - Organelles identified via shape, density
- Cell remains hydrated and frozen.
  - Complements fluorescence techniques

Soft X-Ray Nanotomography of a Yeast Cell



Yeast cell 3-D images
C. Larabell – LBL
Published in Nature (2006)

Recent developments – (beyond Yeast)

Malaria -- Plasmodium falciparum – (~ 0.8 M deaths/Yr)

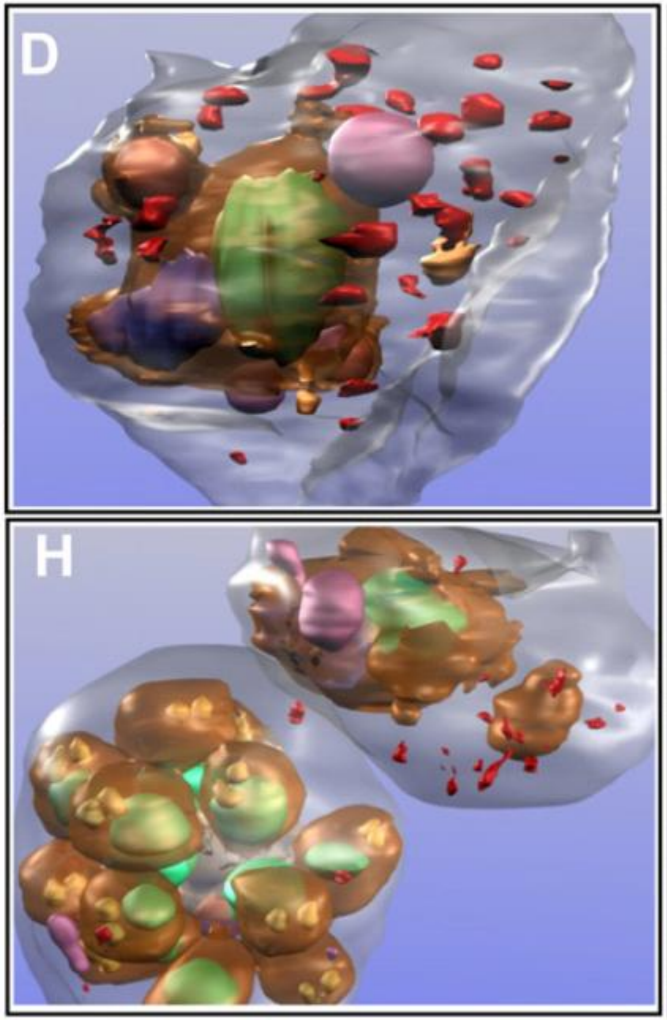
Colonizes red blood cells, consumes hemoglobin

Complex reproductive cycle -- controls host cell chemistry

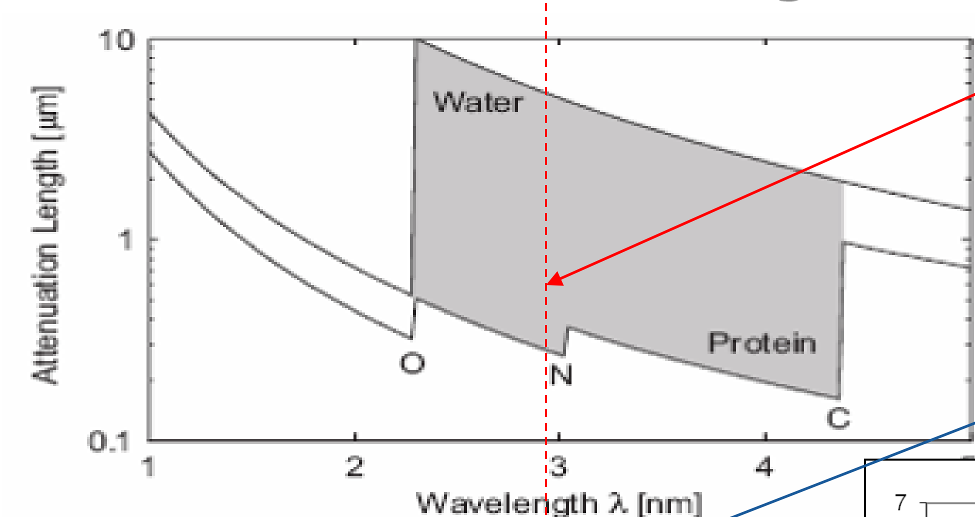
Developing resistance to standard drugs

Soft x-ray tomography follows development of parasite in detail – elucidates dependency of reproductive cycle on cellular hemoglobin

E. Hanssen, C. Knoechel, N. Klonis, N. Abu-Bakar, S. Deed, M. LeGros, C. Larabell, and L. Tilley, "Cryo transmission x-ray imaging of the malaria parasite, p. falciparum," *Journal of Structural Biology*, vol. 173, no. 1, pp. 161-168, Jan. 2011. [Online]. Available: http://dx.doi.org/10.1016/j.jsb.2010.08.013



Lab-based soft x-ray microscopy



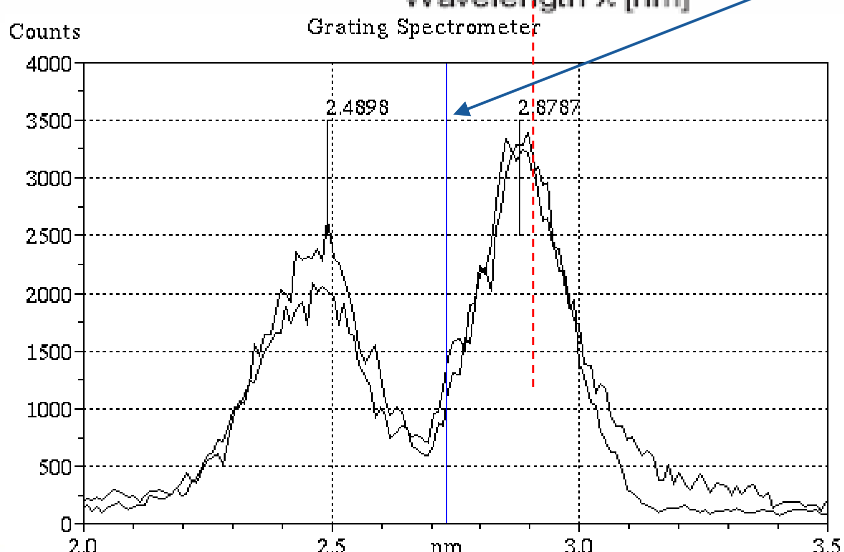
Nitrogen plasma

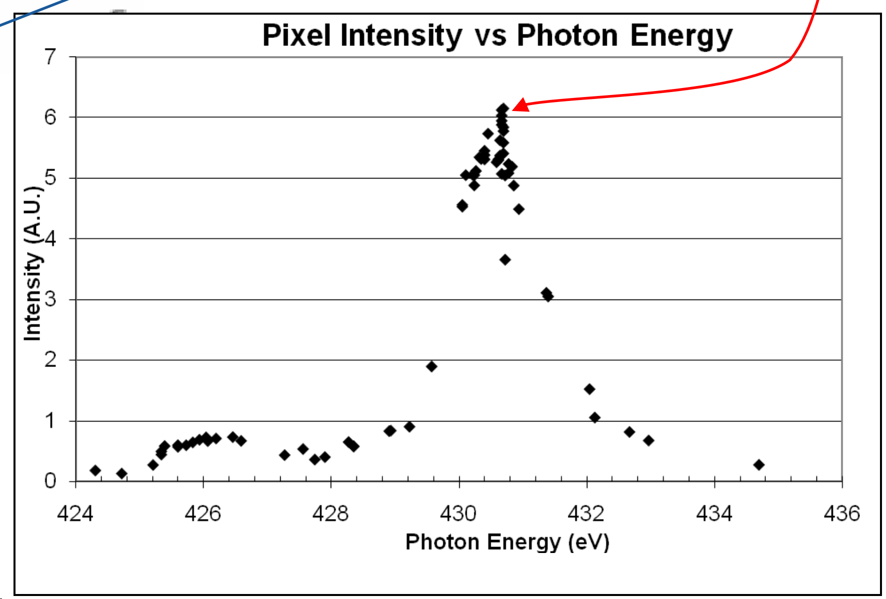
$N^{5+} \Rightarrow$ 2.9 nm ~ 430 eV

Source is slightly modified EQ-10

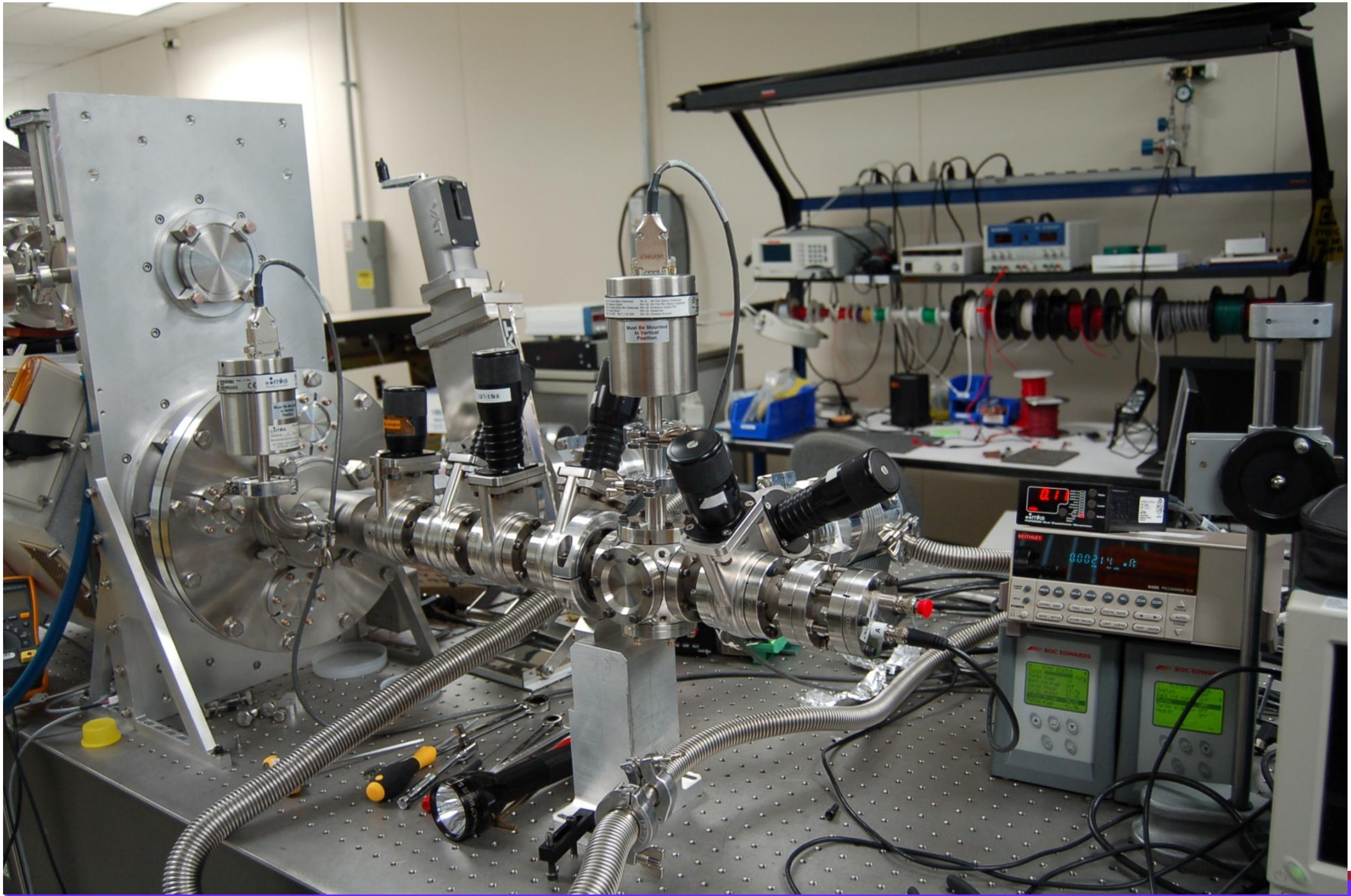
(Nitrogen is molecular & electronegative…)

Extra line at ~500 ev filtered by Titanium foil (L edge at 452 eV)





Laboratory setup



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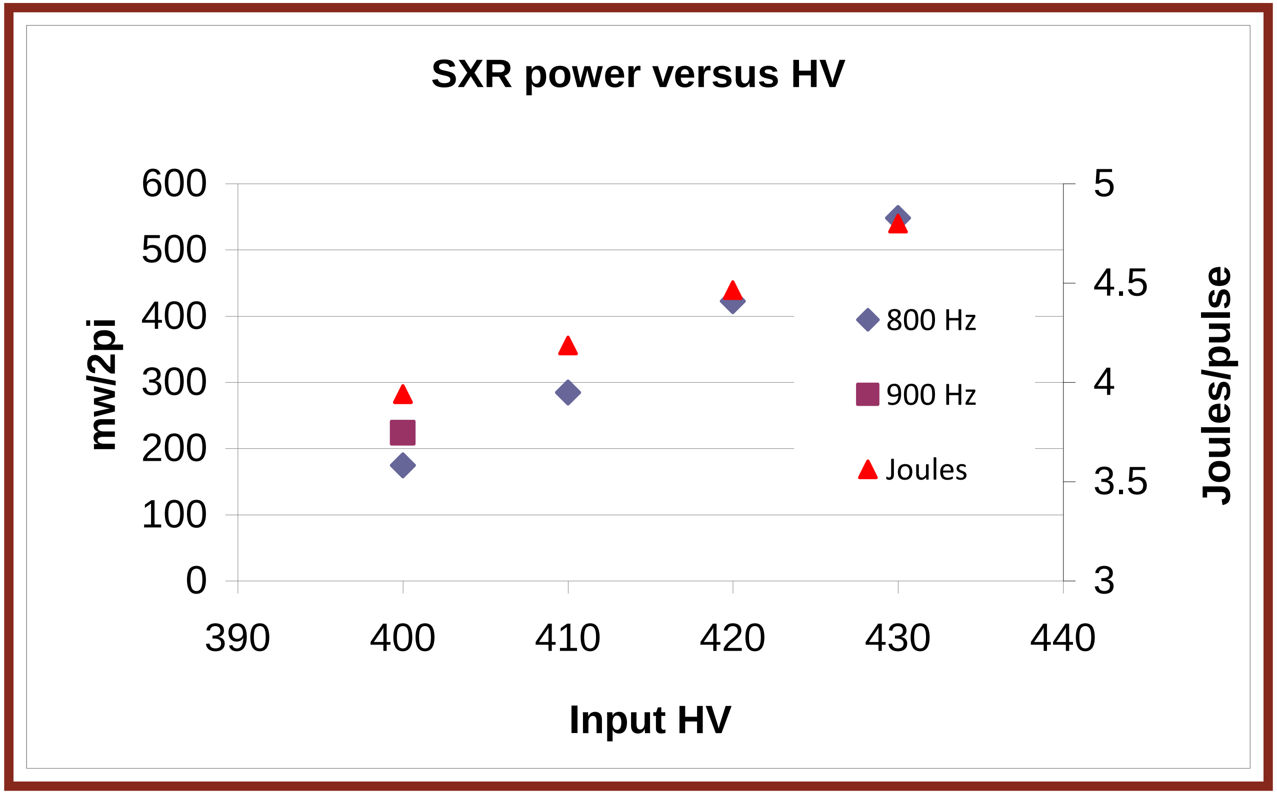
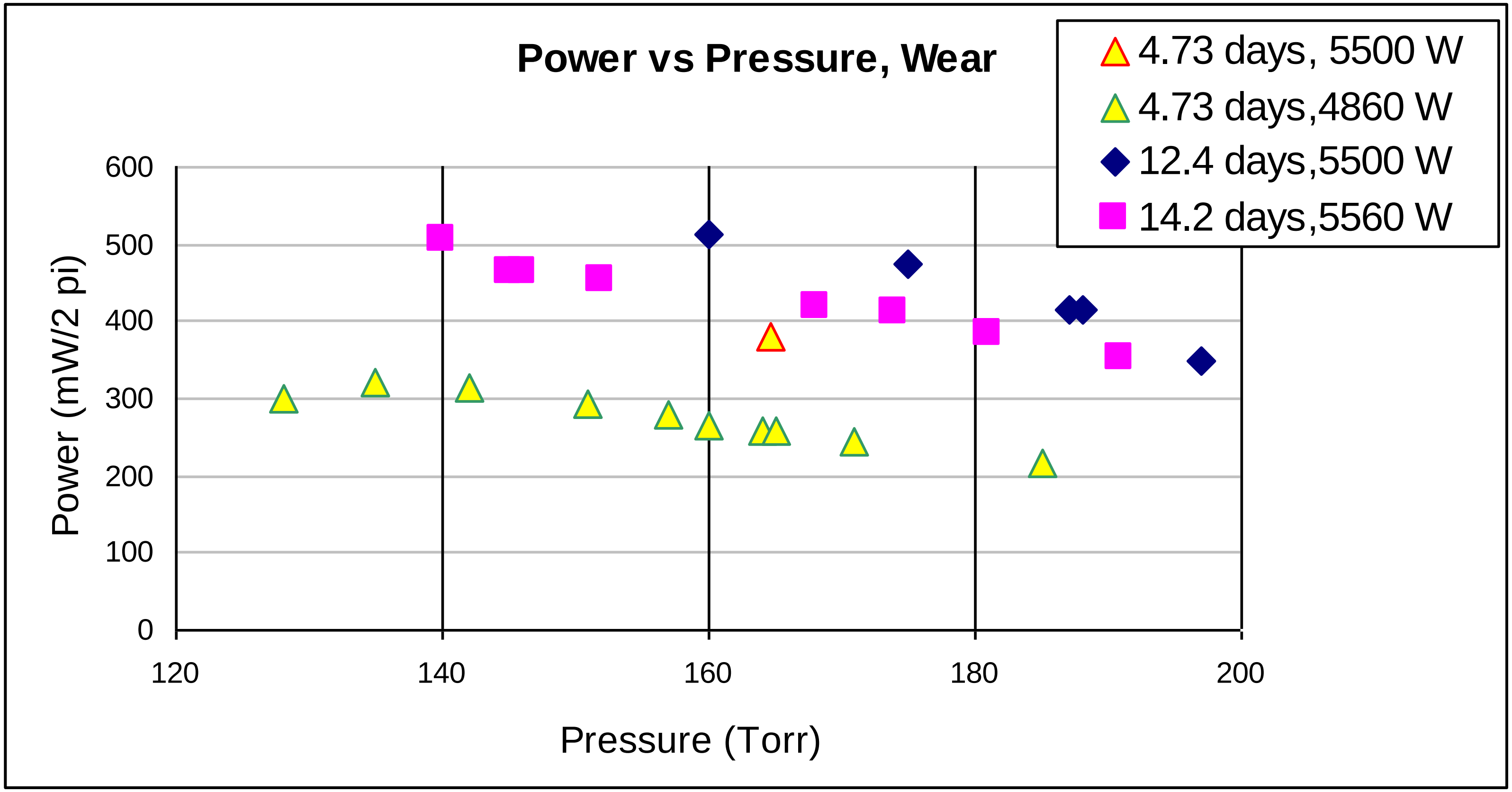
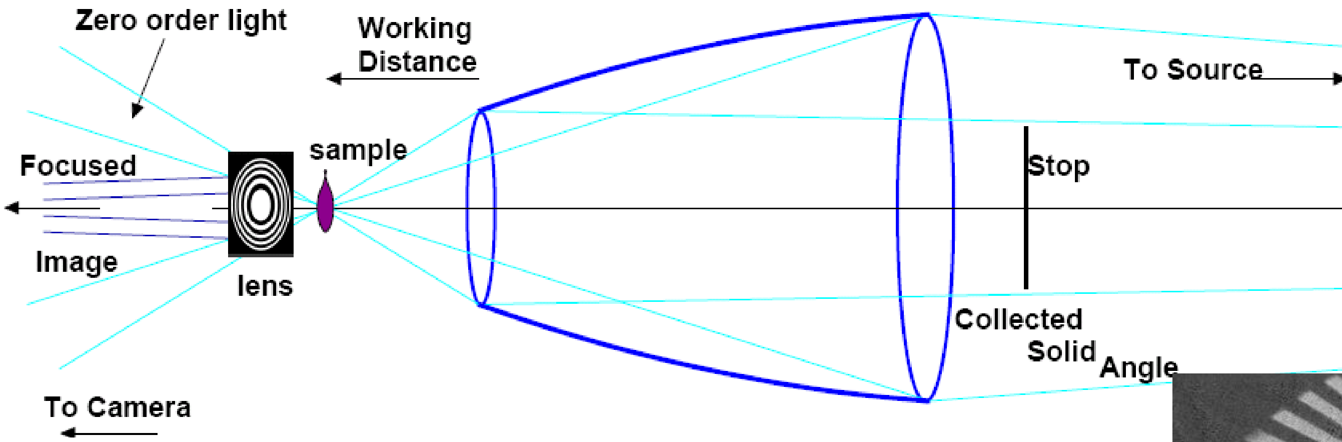
Source performance



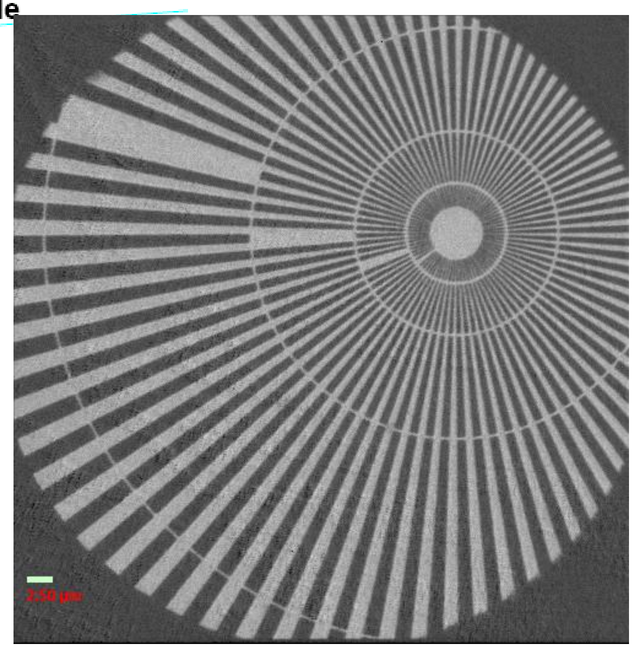
Life testing of optimized system



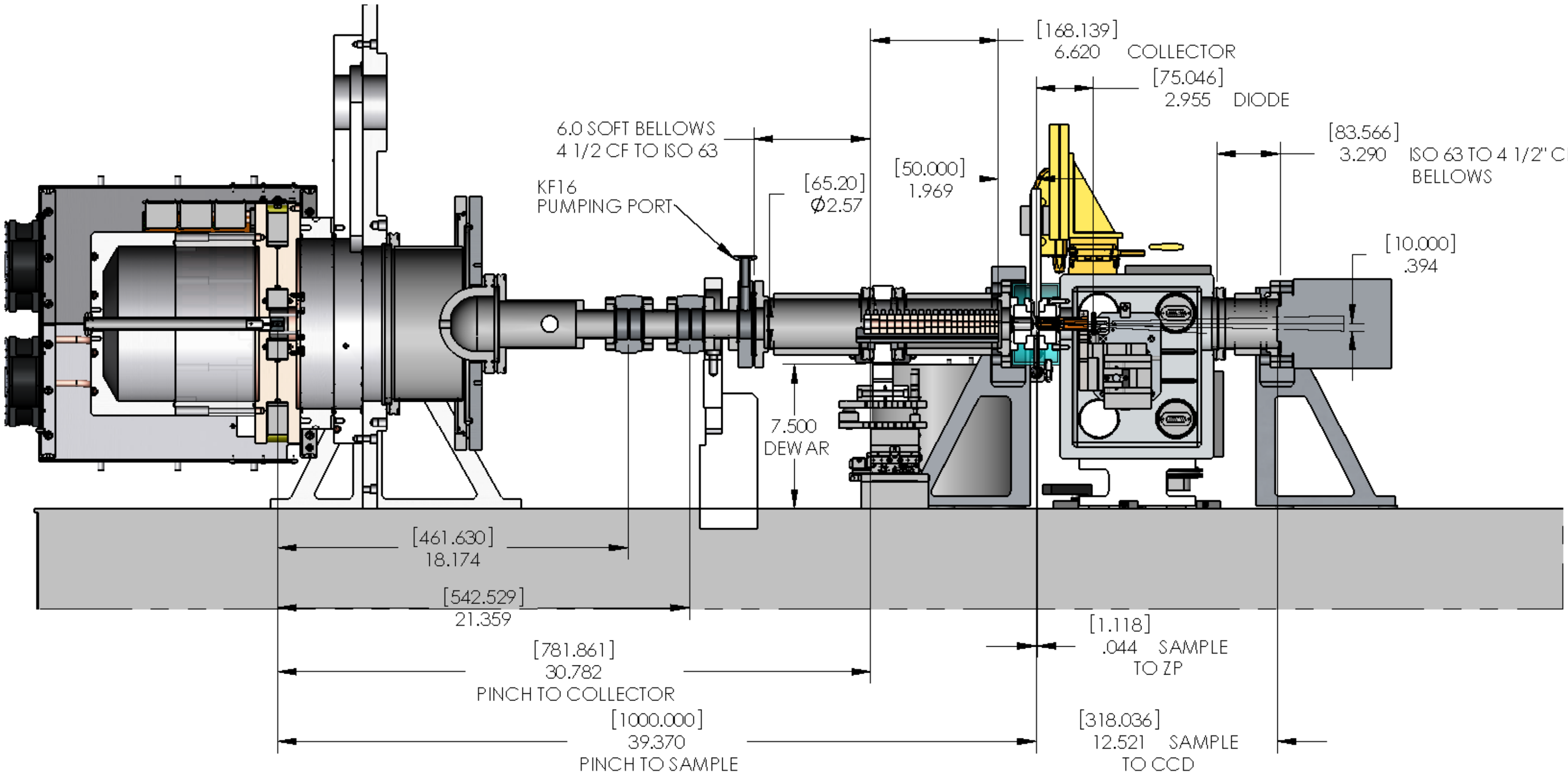
Microscope optical layout



- Zone plate lens has chromatic aberration – requires monochromatic light.
- Resolution achieved in practice (~ 30 nm) implies no issue – source is sufficiently monochromatic; not resolution limiting



Demonstration microscope at Energetiq (NIH Phase II; 5R44RR022488)

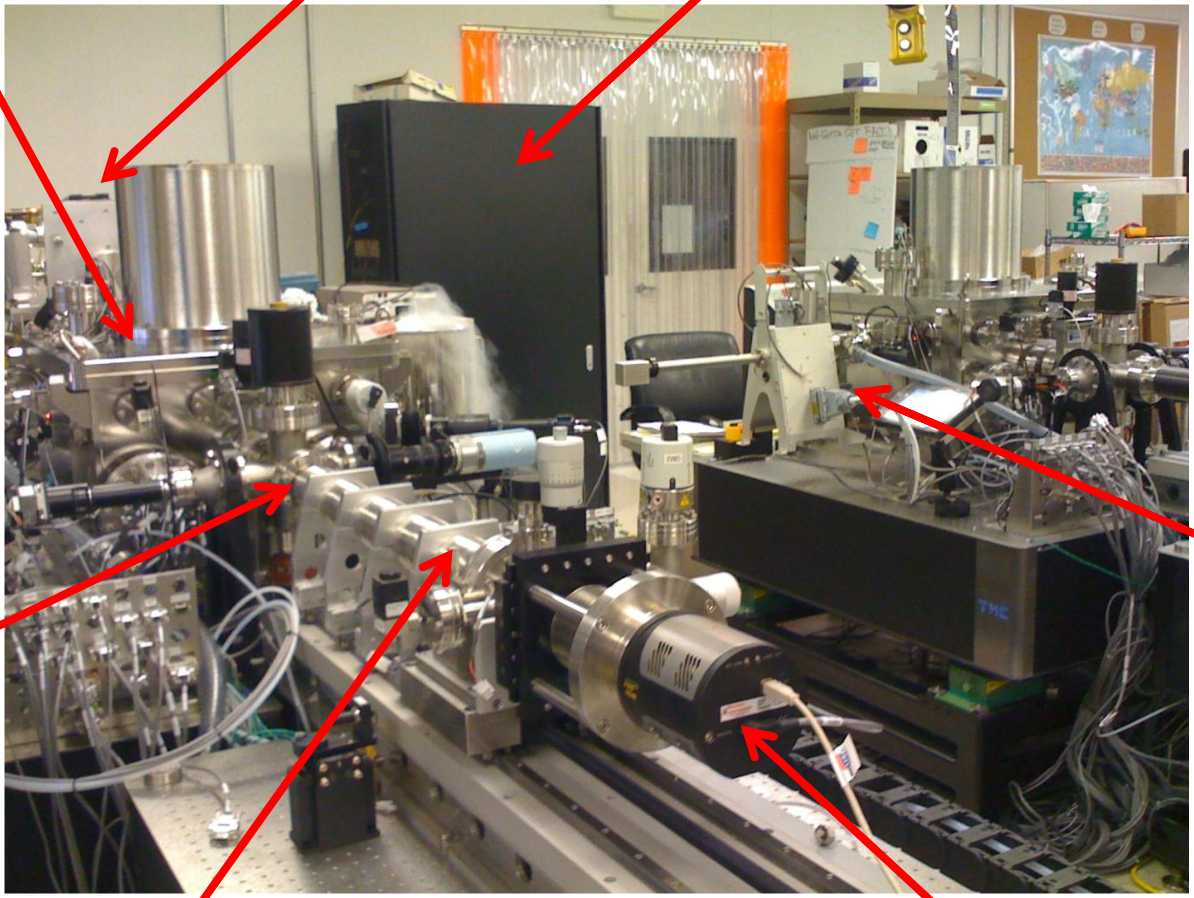


Xradia UltraXRM-L220c Soft X-ray Microscope System

Microscope Chamber

Energetiq EQ-10SXR

Electronics Rack



X-ray Alignment System

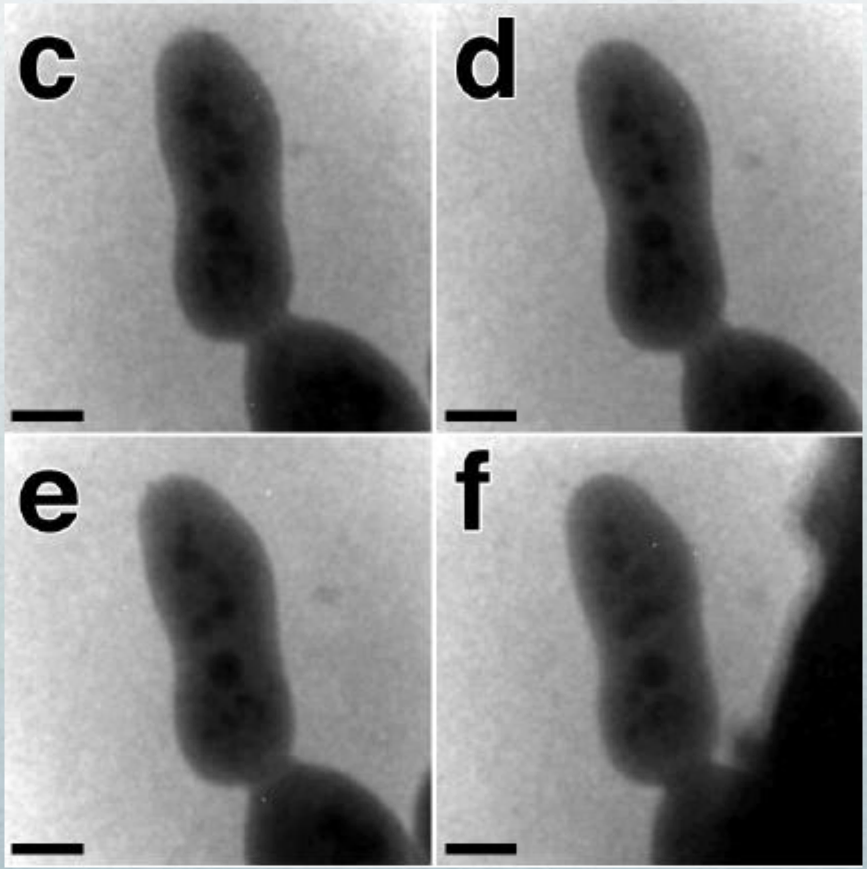
Cryo Sample Transfer System Docked to Microscope

Variable Magnification Slide

CCD Camera

UltraXRM-L220c Usage Example: Imaging procedure

- Images were collected over 121.5 degrees total angular rotation for ~6 hours total integration time.



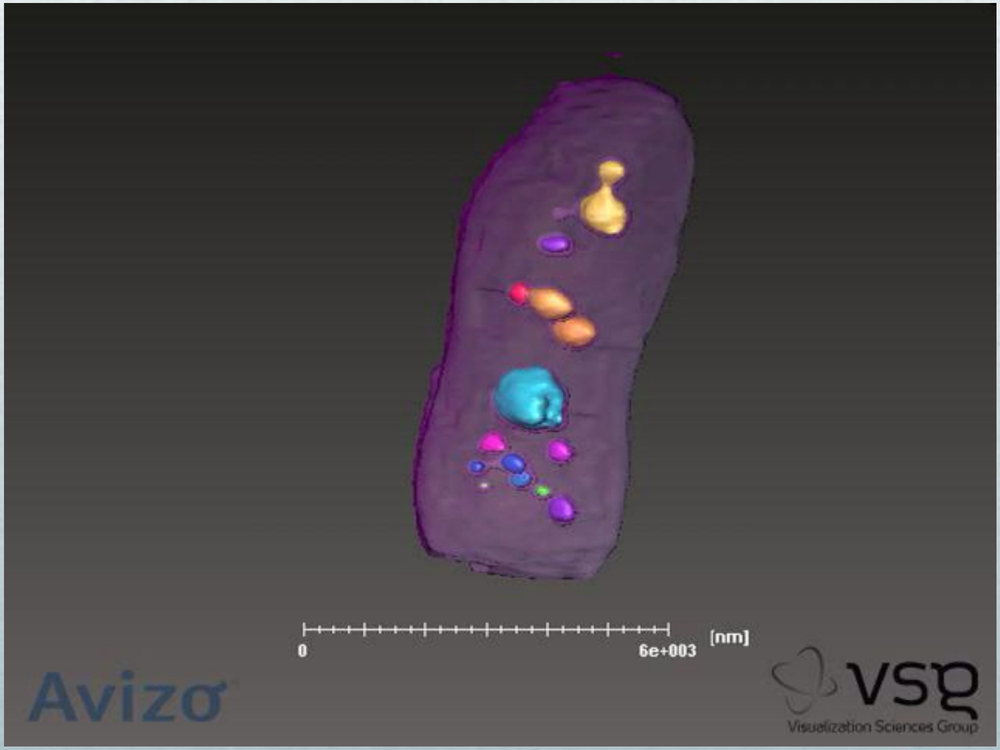
Subset of a radiograph tilt series of one single cell at 0, 20, 40, and 60 degrees tilt angle. Scale bar is 2 μm.

Collaboration with UC Davis. D.B. Carlson et al., submitted for publication

Tilt series movie of the same single cell.

UltraXRM-L220c Usage Example: 3D Rendering/Segmentation

- The single cell was isolated from the virtual volume and then internal features segmented using a watershed algorithm.
- Organelles were then identified by comparing their morphology to that from previous experiments in the literature.



3D Tomography Movie (Segmented)

Collaboration with UC Davis. D.B. Carlson et al., submitted for publication

UC Davis data/paper

Title: Laboratory-Based Cryogenic Soft X-ray Tomography with Correlative Cryo-Light and Electron Microscopy

Authors and Affiliations: David B. Carlson[1], Jeff Gelb[2], Vadim Palshin[2], and James E. Evans[1]

1: Department of Molecular and Cellular Biology, University of California, Davis, Davis, CA 95616, USA

2: Xradia, Inc., Pleasanton, CA 94588, USA

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- **Summary:**
- We present a novel laboratory-based cryogenic soft X-ray microscope for whole cell tomography of frozen hydrated samples. Here, we demonstrate the capabilities of this compact cryogenic microscope by visualizing internal sub-cellular structures of *Saccharomyces cerevisiae* cells. The microscope is shown to achieve better than 50 nm spatial resolution and can image specimens up to 5 micrometers thick. Structures smaller than 100 nm can be detected in the reconstructed tomogram using a cumulative radiation dose of only 7.2 MGy. Furthermore, we establish a direct method for correlative imaging of the exact same unstained yeast cell via cryo-fluorescence light microscopy, cryo-soft x-ray microscopy and cryo-transmission electron microscopy. This completely laboratory-based cryogenic soft x-ray microscope enables greater access to three-dimensional ultrastructure of biological whole cells without chemical fixation, freeze-drying or physical sectioning.

- **Acknowledgements:** J.E.E. and D.B.C. wish to thank Brandon J. Zipp and Ken B. Kaplan for providing access to the yeast strain used in this study for validation purposes. The authors would also like to thank: Pierre Lefebvre for his technical assistance; Energetiq Technology, Inc. for providing the light source with support from grant number 2R44RR022488-04; and Kevin Fahey, Michael Feser and Christian Holzner at Xradia, Inc. for helpful discussions. J.E.E. acknowledges NIH funding support from grant number 5RC1GM91755.

Closing remarks:

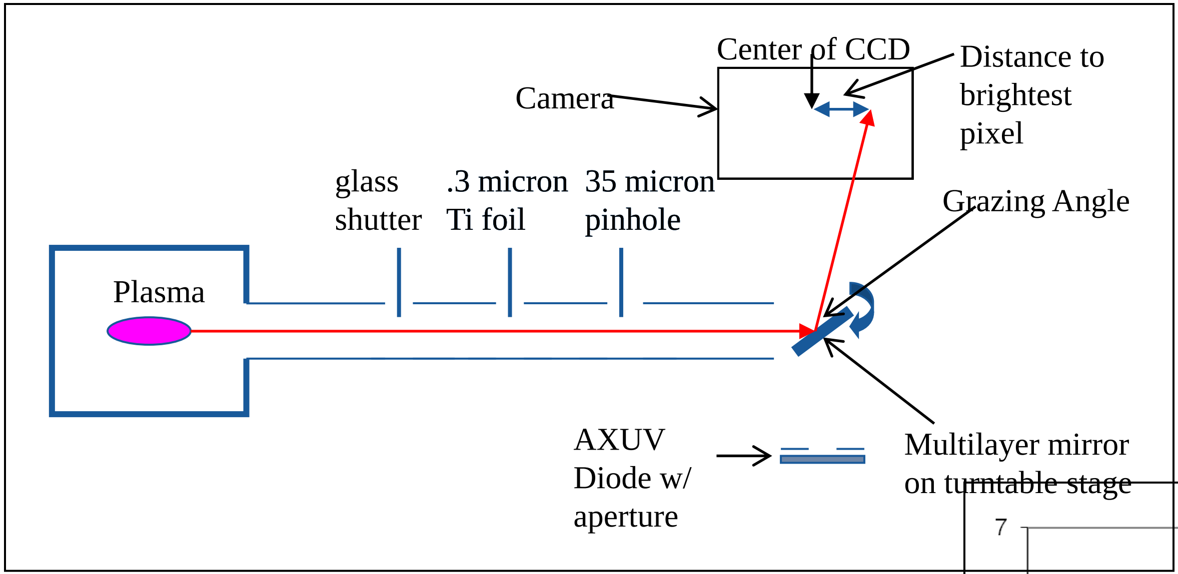
- Small Scale sources allow science that otherwise requires large and/or expensive facilities:
- The Electrode-less Z-pinch source can provide X-rays at 430 eV, with adequate brightness and line quality for zone plate optics – enabling a commercial small-scale microscope.
- Other gasses can be used for other wavelengths – (though we've barely experimented with these…)
  - (eg -- Argon makes ~ 1 W in the range > 300 eV)

Acknowledgements!

- Thank you to valued customers and collaborators
- The team at Energetiq
- The team at Xradia
- NIH grant numbers for Soft X-ray Microscope
  - 5R44RR022488-03, 5R44RR023753-03 and 2R44RR022488-04

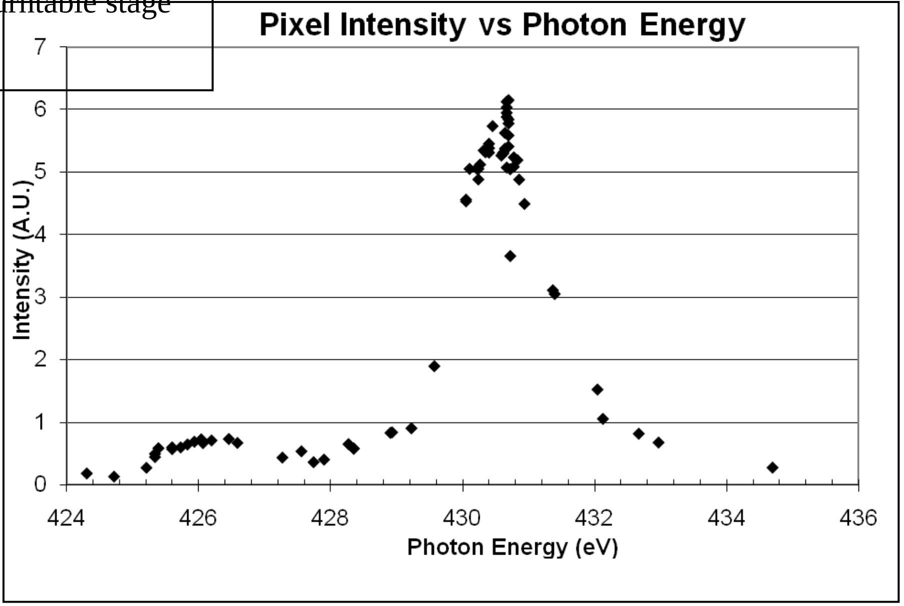
Supplemental Slides

SXR Source Characterization



- Output power
  – 200 to 400 mW / 2pi
- Pinch Size
  – ~800 to 1000 μm

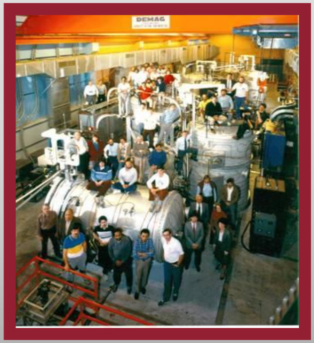
- Used rotatable, graded multilayer mirror as spectrometer and calibrated x-ray diode as a detector
- 'Single' transition (Helium-like)
  - $\Rightarrow$ 'Monochromatic' – allows high quality images with zone plate
  - – z.p. resolution typically < 50 nm



Company Background

Experienced Semiconductor Product Developers

A track record of putting high power plasma devices into production



MIT Plasma Fusion Center

Oxford Plasma Technology

ASTeX, Compact Inst.

MKS Instruments

Energetiq Technology

- List key technologies/products for each?

Simple 'Snowplow' Model Predicts Pinch Time

Based on Krall & Trivelpiece, *Principals of Plasma Physics*
Sheshadri, *Fundamentals of Plasma Physics*

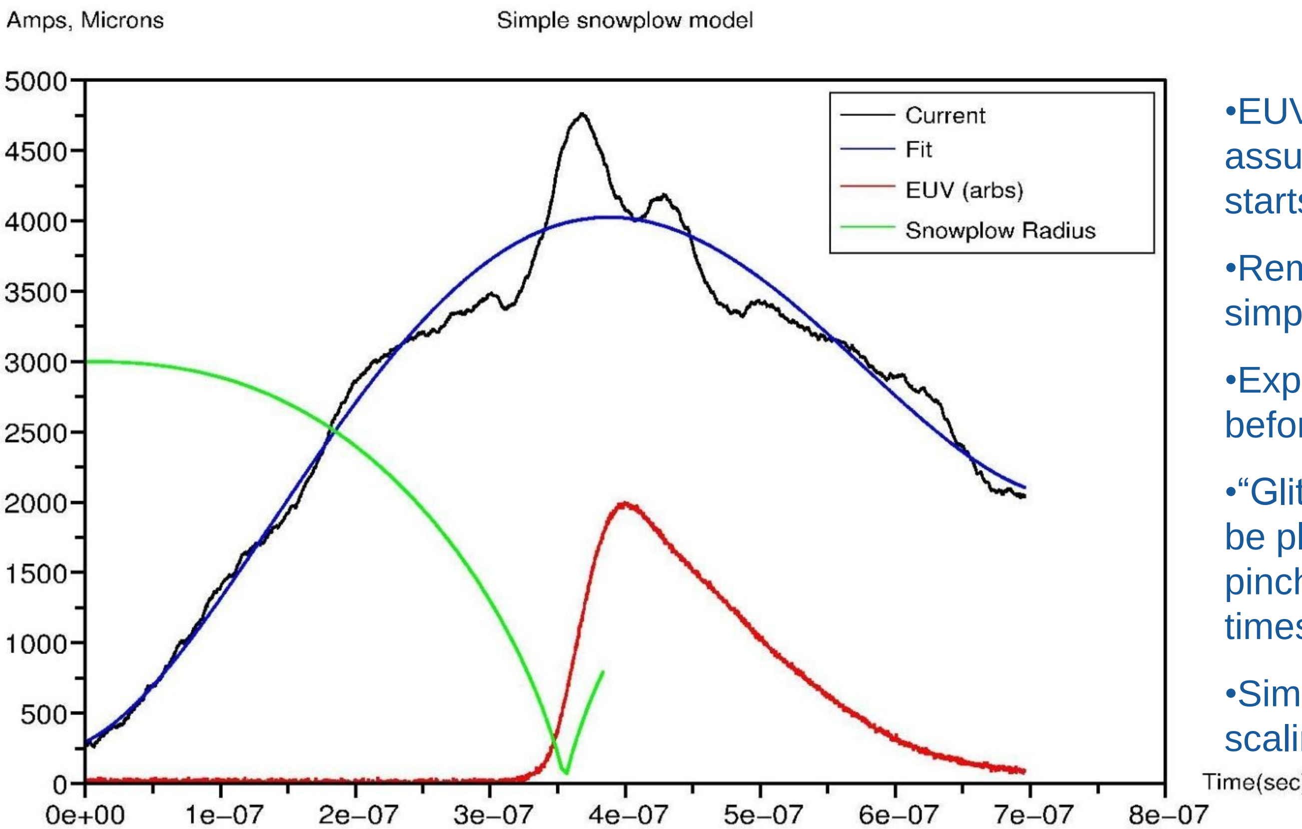
1D model;

- Use measured voltage to estimate plasma current;
- Measured chamber pressure
- Known bore diameter.
- Fit current vs. time by polynomial
- Use fitted current on RHS of snowplow equation

Integrate ...

$$d/dt\left[\pi\rho(r_0^2 - r^2)dr/dt\right] = -\mu_0 \mathrm{I}^2(t)/(4\pi r)$$

Compare model to data:



- EUV output on relatively slow diode – assume pinch occurs where EUV starts to increase.
- Remarkably accurate prediction given simplicity of model
- Experimentally, pinch can occur before or after current peak
- "Glitch" in current at pinch time may be plasma inductance increase due to pinch -- V=d/dt(LI) = 0 on pinch timescale; L goes up, I goes down...
- Simple model may be useful for scaling to higher power

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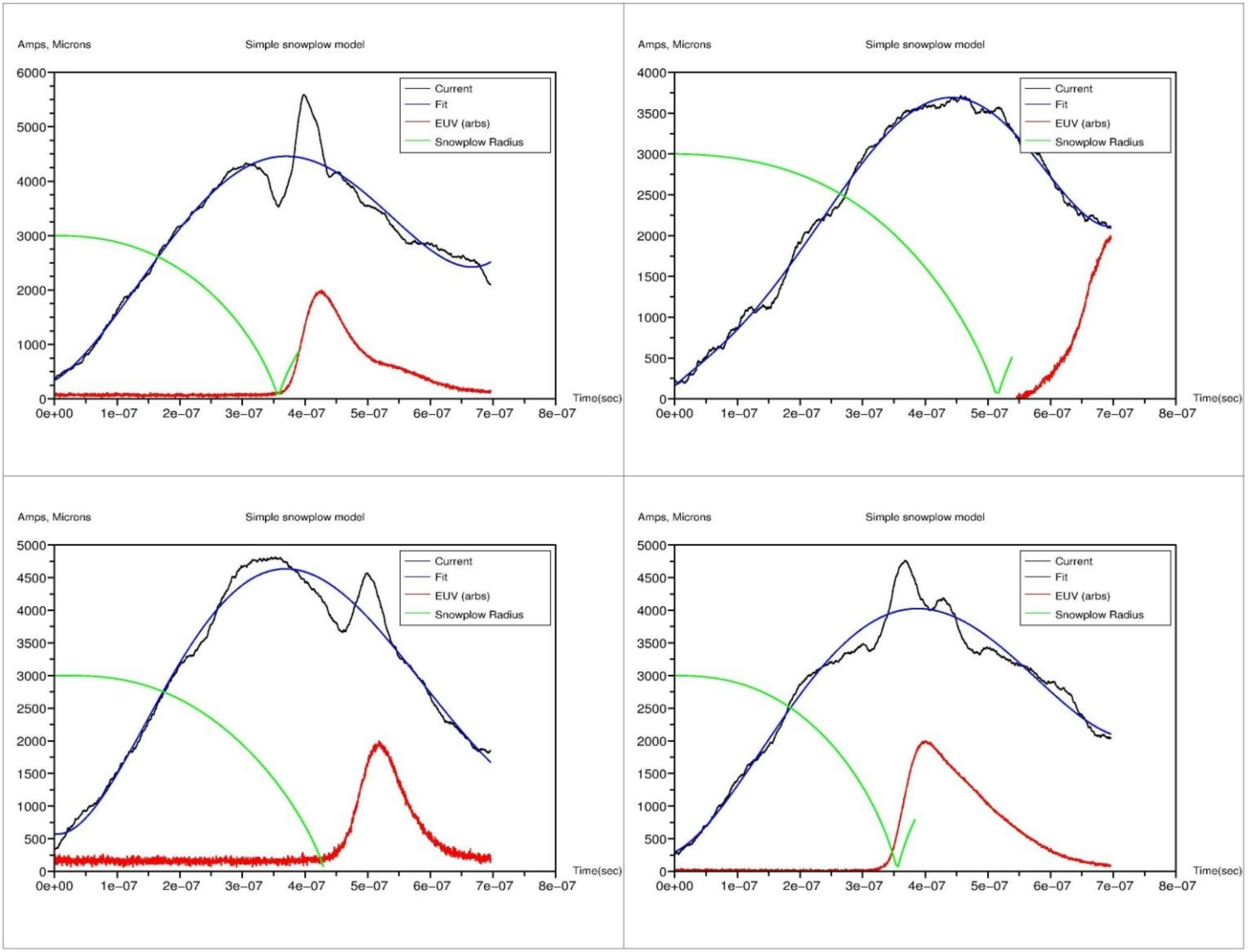

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